once daily intravenously every day but the second). Its weight dropped from 22.8 g to 19.1 g but returned to the pre-injection level 16 days after the last injection. It has since gained satisfactorily. The only other grossly apparent untoward sign was necrosis of one side of the tail probably owing to thrombosis at a site of injection. Control mice receiving saline intravenously recovered their pre-injection weights 6-10 days after the last injection and had no necrosis of the tail. Hematological changes were detected only in the mouse receiving the 6 repeated injections of Na penicillin. The total red-cell count fell to 4,300,000 (counts in control mice were 9,900,000 to 12,600,000) and was still below normal (6,800,000) 16 days after the last injection. A week later the number of erythrocytes per cmm was 9,700,000 and was regarded as normal. The leucocyte count varied considerably (12,000-27,000 per cmm); however, simiiar variations were encountered in control mice. On 2 out of 4 occasions (12 and 23 days after the last injection) there appeared to be an abnormal increase in the proportion of neutrophils (42-56%) at the expense of the lymphocytes which were reduced to 52-36%; the differential counts preceding each of these counts were less abnormal (26% neutrophils, 71% lymphocytes). There were 6-18% neutrophils and 81-93% lymphocytes in the blood smears of control mice.

Summary. Crystalline Na penicillin-G is not a hemolytic agent and is not adsorbed by rabbit erythrocytes suspended in isotonic saline solution. It does not alter the oxygen-consumption of yeast-cells, duck erythrocytes or slices of rat liver suspended in a solution containing 2.05 mg (3400 Oxford units) per ml. A high concentration (0.111M) causes a moderate reduction of the amplitude of each beat of the isolated frog's heart without affecting the rate; the effect is completely reversible. A concentration of 1-5500 in the bath fluid has no significant effect on the isolated ileum or uterus of the guinea pig and does not alter the response of the ileum to acetylcholine or of the uterus to posterior pituitary extract. A single intravenous dose as high as 3,800,000 units (2.32 g) per kilo is tolerated by the mouse as well as a corresponding volume of isotonic saline. One mouse received 6 intravenous injections in 7 days (total dose: 9,800,000 units or 5.93 g per kilo). During the first 2 weeks following injection there was a transient loss of weight and a transient anemia.

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Demonstration of the Properties A, B, M, N and Rh in Red-Cell Stromata.*

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The discovery of the Rh factor in human blood[1,2] and its role in erythroblastosis[3,4] suggests the possibility of preventing the disease by specific desensitization. As a starting material for the preparation of Rh hapten, large quantities of packed red cells are available from blood and plasma banks which have been established in most of the large hospitals.

The intact erythrocytes contain only relatively minute quantities of the group-specific substances, A, B, M, N, and Rh, so that the therapeutic injection of whole blood would undoubtedly be ineffectual unless large quantities were injected. Such a procedure would

* Aided by a grant from the United Hospital Fund of New York City.

1 Landsteiner, K., and Wiener, A. S., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 343.

2 Landsteiner, K., and Wiener, A. S., *J. Exp. Med.*, 1941, **74**, 309.

3 Levine, P., Katzin, E. M., and Burnham, L., *J. A. M. A.*, 1941, **116**, 825.

4 Levine, P., Burnham, L., Katzin, E. M. and Vogel, P., *Am. J. Obst. and Gyn.*, 1941, **42**, 925.

not be without danger, especially since the injection of large amounts of hemoglobin may cause dangerous renal complications. No method has yet been devised for extracting the group substances from erythrocytes in a purified form. However, by converting the red cells into stromata, the bulk can be considerably reduced and such red-cell stromata might find therapeutic application until methods are devised for the extraction of the individual blood properties in a pure form. The purpose of our study was to ascertain whether the process of converting intact red cells into stromata damaged the blood properties. If no damage occurred, one would expect an increase in the concentration of the group substances approximately equal to the reduction in volume resulting from the conversion of the red cells to stromata.

As a rule, the packed cells from a pint of blood were processed at one time; that is, we started with approximately 250 cc of cells. The cells were diluted to about 2 liters with saline solution and the suspension passed through a steam-driven Sharples Super-Centrifuge at approximately 200 cc per minute, with steam pressure of 10 lbs (approximately 10,000 rpm). The packed cells were resuspended in 2 liters of fresh saline and the process repeated. Where the washings did not appear almost colorless, the process would be repeated a third time. The packed cells were then diluted with 2 liters of distilled water, and after a detention period of 30 minutes the suspension was run through a Travis Colloid Mill. After this treatment the stromata were reduced to approximately uniform, minute particles, just visible under the high dry power of the microscope.[5] The particles were deposited by passing the suspension through the Sharples centrifuge, prepared with a cellophane liner, at a rate of 50 cc a minute, under steam pressure of 38 lb (50,000 rpm). The maximum yield was 10 cc of a semi-solid substance, which was light brown in color with a pink cast. With this technic the amount of group substance lost in the washings was apparently insignificant, as determined by quantitative inhibition tests on samples of the supernatants.

Tests were carried out to compare the concentration of group substances A and B in the original packed erythrocytes, red-cell stromata, and saliva of the corresponding group from secretors, by the inhibition or absorption method described by Wiener and Kosofsky.[6] Similar comparative tests were carried out on red cells and red-cell stromata for the properties M, N, and Rh.

The technic used in these experiments may be briefly described as follows: Serial dilutions (undiluted, $1:2^1$, $1:2^2$, $1:2^3$, etc) of packed red cells, red-cell stromata or saliva were prepared and one drop of each dilution of the material tested was placed in a series of test-tubes. A saline control was included in each series. A drop of a fixed dilution of the appropriate testing serum was added to each tube and the mixtures were allowed to react for one hour at room temperature. In the tests on the packed red cells, the supernatant fluids were transferred to another series of tubes at the end of the period of fixation, and a drop of the appropriate test cells was added to each tube. In the tests on stromata and saliva, the test cells were added directly to the original mixtures. One hour after the test cells had been added, the reactions were read, the absorption or inhibition titer being the last dilution of the material being tested which inhibited the agglutination of the test cells. For example, the first absorption titer in Table I is 2^6 or 64, indicating that this was the highest dilution of the packed red cells of group A_1 which completely absorbed the anti-A agglutinins in the group B testing serum. The reactions were usually very smooth, so that, for instance, in the case described above, dilution 2^7 gave a weak positive reading, while higher dilutions gave progressively stronger reactions, dilutions beyond 2^9 or 2^{10} giving as strong reactions as the saline control.

With regard to the testing sera, the group A and group B sera were produced in blood donors by isoimmunization and were selected

5 *Cf.* Sigurdsson, B., *J. Exp. Med.*, 1943, **77**, 4.

6 Wiener, A. S., and Kosofsky, I., *J. Immunol.*, 1941, **41**, 413.

TABLE I.
Comparison of Titers of Group Substances A, B, M, N, and Rh in Packed Red Cells and Red-Cell Stromata.

Group Substance	Material tested		Test Sera	Test Cells	Inhibition or Absorption Titer
A	Group A_1	cells	Group B	Group A_2	26
			" A	" B	0
		stromata	" B	" A_2	211
			" A	" B	0
		saliva	" B	" A_2	210
			" A	" B	0
B	Group B	cells	Group B	Group A_2	0
			" A	" B	25
		stromata	" B	" A_2	0
			" A	" B	28
		saliva	" B	" A_2	0
			" A	" B	29
M	Type M	cells	Anti M	Type M	20
		stromata	"	"	22
	Type MN	cells	"	"	21
		stromata	"	"	22
	Type N	stromata	"	"	0
N	Type N	cells	Anti-N	Type N	20
		stromata	"	"	21
	Type MN	cells	"	"	0
		stromata	"	"	0
	Type M	stromata	"	"	0
Rh_o	Type Rh_1	cells	Anti-Rh_o	Type Rh_1Rh_2	21
		stromata	"	"	25
	Type Rh_2	cells	"	"	20
		stromata	"	"	25
Rh′	Type Rh_1	cells	Anti-Rh′	Type Rh_1	20
		stromata	"	"	24
	Type Rh_2	cells	"	"	0
		stromata	"	"	0
Rh″	Type Rh_1	cells	Anti-Rh″	Type Rh_2	0
		stromata	"	"	0
	Type Rh_2	cells	"	"	20
		stromata	"	"	24

because of the ease with which the antibodies were neutralized in inhibition tests. The group A serum had a titer of 150 and was used in the tests in a dilution of 1:15; the group B serum had a titer of 600 and was used in a dilution of 1:20. The anti-M testing fluid had a titer of 15 and was used in a dilution of 1:3; the anti-N testing fluid had a titer of 8 and was used undiluted. All 3 varieties of anti-Rh sera used (anti-Rh_o, anti-Rh′, and anti-Rh″)† had titers of approximately 50 and were used in a dilution of 1:5. These were the same antisera which have already been described in detail in a previous publication.[7]

The results of a number of these experiments have been combined and summarized in Table I. It will be seen that the titers

† The designations of the Rh types and antisera correspond to the latest improved nomenclature proposed by Wiener (*Science*, 1944, **99**, 532).

[7] Wiener, A. S., Sonn, E. B., and Belkin, R. B., *J. Exp. Med.*, 1944, **79**, 235.

of the red-cell stromata for properties A, B, Rh_o, Rh′, and Rh″ are approximately 8 to 32 times as high as the corresponding titers of the original red cells. When one considers that 250 cc of red cells yield about 10 cc of red-cell stromata, the results are in satisfactory agreement with the expectations. In the case of the M and N properties, on the other hand, the expected rise in titer was not obtained, though there was in general a slight increase. This suggests that the processing damages the M and N properties.‡

It might be mentioned that the inhibition and absorption titers obtained for the various Rh properties have been consistently lower than the titers obtained for A and B. While it is true that it is not possible to compare the reactions of two antisera of different specificities, still these findings suggest that the number of Rh haptens per red cell may be less than the number of A, B, or O haptens. If this is proved to be correct, it might furnish an explanation for some of the peculiarities of the Rh *in vitro* reactions.

Red-cell stromata prepared with sterile precautions have been injected intramuscularly into human volunteers, without systemic or local reactions of any kind; also the intravenous injection of large quantities of stromata into rabbits appears to be innocuous, provided these are prepared with sterile precautions and smooth emulsions containing no coarse particles are injected. It would appear, therefore, that the clinical trial of the material for attempting the desensitization of Rh-negative mothers of erythroblastotic fetuses may be justified.

Summary. From 250 cc of packed erythrocytes approximately 10 cc of red-cell stromata can be obtained. The titers of the group substances, A, B, and Rh, in the stromata are almost proportionately higher than the corresponding titers of the original erythrocytes. However, the calculated rise in titer was not obtained for properties M and N, suggesting that these may have been damaged by the processing of the cells. The possible use of red-cell stromata for treating sensitized Rh-negative individuals is discussed.

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‡ However, E. Witebsky and A. Heide (*Arch. Path.*, 1940, **30**, 1154) have reported the production of anti-N sera by injecting rabbits with boiled stromata of type N.

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Production and Implications of an Antiserum to Necrosin.*

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The writer has recently demonstrated that the pattern of injury in acute inflammation seems to be primarily referable to the liberation of a factor located in the euglobulin fraction of exudates.[1, 2] This factor has been termed necrosin. It is either a euglobulin or at least it seems to be associated with that fraction of exudates, *i.e.*, as obtained by precipitation with $(NH_4)_2SO_4$ at one third saturation. In addition, this fraction manifests pyrogenic and leukopenic properties.[2]

It occurred to the writer in connection with these studies that an antiserum to necrosin, if obtainable, may prove to be of theoretical interest and possibly also of clinical value. For this reason, in the fall of 1942, a series

* This is paper XXVIII of a series entitled "Studies on Inflammation."

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1 Menkin, V., *Science*, 1943, **97**, 165.

2 Menkin, V., *Arch. Path.*, 1943, **36**, 269.